Methods: Prospective, randomized, double-blind, parallel group, placebo-controlled 3-year trial. Patients with primary hip OA (ACR criteria) were randomly assigned to receive either 300 mg/day of ASU (n: 189) or placebo (n: 209). Patients ≥ 45 years, symptomatic (painful ≥ 1 year, Lequesne index ≥ 3) with a minimum joint space width (JSW) of the target hip between 1-4 mm on a pelvic radiograph were randomized into 2 strata, based on baseline JSW: < 2.5 or ≥ 2.5 mm. 3 standing radiographs were taken annually: pelvis, target hip anteroposterior (AP), and oblique views. JSW was measured at the narrowest point on pelvic or target hip AP view by manual measurement using a 0.1 mm-graduated magnifying glass. This method was deemed the most sensitive to change in a pilot study and the best of 2 readers was selected prior to unblinding. The primary outcome was based on the loss in the narrowest JSW over 3 years. *Statistics:* All patients with at least 2 radiographs of the same view during the trial were included in the Full Analysis dataSet (FAS) population. An Analysis of Covariance Mixed Model for Repeated Measurements (MMRM) was performed and Missing At Random (MAR) used to handle missing data. Minimum adjusted JSW change was compared between groups by a continuous approach: 3-year JS narrowing mean in mm and a more robust analysis: comparison fo the percentage of "progressors" defined by a JSW loss ≥0.5 mm (based on the smallest detectable change of the reader [1]).
Results: 499 patients were selected, 399 randomized and 345 available for the FAS (166 ASU; 179 placebo group). Baseline demographic and hip OA characteristics were similar in both groups. Patients were aged 62 (8) years, 54% were women, mean BMI was 27 (4), mean symptom duration 4 years (5), 0-100 normalised Lequesne index 30 (9), VAS global pain 37 (23) mm. Mean baseline JSW was 2.8 (0.9) mm in both groups. 166 patients discontinued the study, 55% of these for inefficacy (74 were planned for total joint replacement). Results of mean JSW loss and % of progressors at year 3 are in Table 1.

Table 1. Results of the analysis on JS change

	% Progressors			Mean JS loss (sem)		
Population	ASU, N=166	Placebo, N=179	Cochrane Mantel Haenzel P adjusted on stratus	ASU	Placebo	P
Total	40%	50%	0.039	-0.64 (0.07)	-0.67 (0.06)	0.72

sem: standard error of the mean.

Although no significant intergroup difference was observed on the adjusted mean JSW loss, the number of progressors was 20% lower in the ASU (p = 0.039). ASU were well tolerated in this study.
Conclusions: This study shows that 3-year treatment by ASU appears to reduce the percentage of JSW deteriorating patients compared to placebo, indicating a potential structure-modifying effect of ASU in hip OA. The clinical relevance of these encouraging radiographic results requires further assessment.

Reference

[1] Ornetti P. OAC 2009;17:842-9.

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INHIBITION OF PROSTAGLANDIN E_2 SYNTHESIS BY HYALURONAN THROUGH NF-κB DOWN-REGULATION VIA CD44 IN U937 MACROPHAGES

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Purpose: Prostaglandin E_2 (PGE_2) is one of the key mediators of inflammation in rheumatoid arthritis (RA) joints. Intra-articular injection of high molecular weight hyaluronan (HA) into RA knee joints relieves arthritic pain. Although HA has been shown to inhibit PGE_2 production in cytokine-stimulated synovial fibroblasts, it remains unclear how HA suppresses PGE_2 production in catabolically activated cells. Furthermore, HA effect on macrophages has rarely been investigated in spite of their contribution to RA joint pathology. This study was aimed to investigate the inhibitory mechanism of HA on lipopolysaccharide (LPS)-stimulated PGE_2 in U937 human macrophage culture system, which is a useful tool for experiments on HA effect.
Methods: 1. U937 cells were treated with PMA for differentiation into macrophages. With or without pretreatment with one of HA, NS-398, and BAY11-7085, the cells were stimulated with LPS. In another set of experiments, the cells were incubated with anti-CD44 antibody or non-specific IgG before pretreatment with HA.
2. PGE_2 concentrations of the cell-free supernatants were determined using an enzyme-linked immunosorbent assay.
3. The cell lysates and nuclear extracts were prepared for immunoblot analysis.
4. HA binding to CD44 was evaluated by fluorescence microscopic analysis.
Results: Stimulation of U937 macrophages with LPS enhanced PGE_2 production in association with increased protein levels of cyclooxygenase-2 (COX-2). Pretreatment with HA of 2,700 kDa resulted in suppression of LPS-induced COX-2, leading to a decrease in PGE_2 production. While LPS activated NF-κB pathway, inhibition studies revealed the requirement of NF-κB for LPS-stimulated PGE_2 production. HA down-regulated the phosphorylation and nuclear translocation of NF-κB by LPS. Fluorescence cytochemistry demonstrated that HA bound to CD44, the principal HA receptor, on U937 macrophages. Anti-CD44 antibody reversed the inhibitory effects of HA on LPS-activated PGE_2, COX-2, and NF-κB.
Conclusions: These results clearly demonstrated that HA of physiological molecular weight suppressed LPS-stimulated PGE_2 production via CD44 through down-regulation of NF-κB. Clinical administration of high molecular weight HA into RA joints may decrease PGE_2 production by activated macrophages, which could result in improvement of arthritic pain.

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THE CINOD NCX 429 INHIBITS MICROVASCULAR INFLAMMATION THROUGH ITS NO-RELEASING PROPERTIES

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Purpose: Cyclooxygenase-inhibiting nitric oxide (NO) donators (CINOD) are novel anti-inflammatory drugs in which NO donation is aimed at mitigating the side effects of non steroidal anti-inflammatory drugs (NSAIDs). In particular, naproxcinod, the first-in-class CINOD, showed favorable effects on systemic blood pressure, which is often elevated in elderly patients suffering from osteoarthritis (OA) or rheumatoid arthritis (RA). The present study was undertaken to assess the NO-mediated effects of NCX 429 in the inflamed microvasculature.
Methods: Intravital microscopy (IVM) of the mouse mesentery was applied to study fluid (shear stress) and inflammatory (leukocyte adhesion) events in male C57bl6 mice treated with L-NAME (2 mg/ml in the drinking water for 5 days). NCX 429 (1.7-5.7-17 mg/kg) or naproxen (10 mg/kg, a dose equivalent to the highest dose of NCX 429) was given orally 1 h prior to 30 min observation of the microvasculature. The R-enantiomer of NCX 429 (NCX 1351, 5.7 mg/kg), which does not inhibit cyclooxygenase (COX) but retains NO-donating properties, was also tested. In a separate set of experiments, the effect of IL-1 beta treatment (10 ng, i.p.) on top of L-NAME chronic exposure was tested, giving the cytokines 2 h prior to observation. All data are mean ± SEM of 5 mice per group.
Results: Addition of L-NAME in the drinking water augmented shear stress in the mesenteric post-capillary venules from ~450 to 750 s^{-1} ($p<0.05$). NCX 429 corrected this alteration at all doses tested. A similar effect was observed on cell adhesion (data at 15 min): from 2.0±0.2 in control, to 4.8±0.3 and 2.1±0.1 adherent cells in L-NAME and L-NAME + NCX 429 (1.7 mg/kg), respectively ($p<0.01$). NCX 1351 was also effective on shear stress, while naproxen altered neither shear stress nor cell adhesion. Addition of IL-1 beta on top of L-NAME modified the microvascular status, bringing shear stress back to control (untreated) values (475±20 s^{-1}) and markedly increasing cell adhesion (12.5±3.5 cells per post-capillary venule). Shear stress was not affected by any of the pharmacological treatments. IL-1 beta-induced cell adhesion was effectively inhibited by NCX 429 even at the lowest dose tested of 1.7 mg/kg (1.9±0.3 adherent cells; $p<0.01$ *vs.* L-NAME + IL-1 beta), whereas naproxen only partially (~50%) reduced cell adhesion (7.0±0.7 cells per mouse; $p<0.05$ *vs.* L-NAME + IL-1 beta).
Conclusions: Removing endogenous production of NO alters microvascular events in a way that is corrected by NCX 429. These effects, apparently independent from COX inhibition, are particularly evident with respect to shear stress (plausibly resulting from upstream vasoconstriction) and cell adhesion (due to increased endothelial adhesiveness properties). Addition of IL-1 beta (inflammatory stimulus) on top of L-NAME unveiled anti-inflammatory properties of naproxen, though NCX 429 was still more potent and effective. Altogether these results widen our knowledge on the properties of NCX 429 (and possibly other CINODs) and provide supporting evidence for the favorable effects of CINODs on the vasculature.